## REMARKS

The Applicants thank the Examiner for the careful analysis of the application and claims. The Applicants present the forgoing amendments and the following arguments and submit that the case is now in a proper form for allowance.

*Informalities.* The Examiner has objected to an informality in the previous amendment and requested that a new marked up version be sent with this response. The Applicants contacted the Examiner on December 17, 2003 requesting further details on the version of claims that should be marked up in the present response. No response was received from the Examiner. The Applicants have marked up the claims to reflect the changes from the most recently submitted set of claims in the response to the restriction requirement. If the Examiner would like the Applicants to submit a marked up version of the claims showing the changes from the original claims to those submitted with the response to the restriction requirement or with this response, one will be provided upon request.

*Sequence listing.* The Examiner has stated that the application does not contain a proper sequence listing as the sequences contained in Figure 3 were not included in the listing. The Applicants further noted sequences in Figure 7 that were not included in the original sequence listing. The Applicants have enclosed a new sequence listing, both paper and electronic versions, with a statement that the sequence listing contains no new matter. All of the sequences in the listing were disclosed in the parent application. Modification of the format in which the sequence are presented to facilitate searching does not constitute new matter. Seq ID numbers have been inserted into the specification as noted above to refer to the sequences in Figures 3 and 7. The Applicants submit that this inclusion of Seq ID numbers in the text does not constitute new matter as it is simply a pointer to another existing portion of the specification. The Applicants submit that the

sequence listing is now complete and in proper form for allowance.

*Drawings.* The Examiner has objected to the drawings for not containing Seq ID numbers. The Examiner stated that the drawing correction could be effected by modification of the figure legend. The Applicants have amended the figure legend for Figures 3 and 7 as shown above. Therefore, the objection is overcome.

*Election/restriction.* The Examiner has acknowledged the election made in the restriction and has included the new claims 11-13 in the group under examination. The Applicants submit that the newly added claims 14-15 also fall within the scope of the invention as limited in the restriction requirement.

*Claim objection.* The Examiner has objected to claim 7 for containing an informality. The Applicants have amended the claim to insert "that" after -- protein--. The Applicants submit that the amendment overcomes the objection.

*Rejection under 35 U.S.C. §112, ¶1.* The Examiner has rejected to claims 4-8 and 11-13 under 35 U.S.C. 112, paragraph 1 for containing matter that was not fully described in the specification. The Applicants submit that all of the limitations of the claims are supported both in the instant application and in the provisional patent application on which the instant application is based.

To insure uniformity with the provisional patent application document that would be available to the Examiner, the Applicants ordered a certified copy of the provisional patent application. As the pages in the original filing were not numbered, the Applicants have numbered the pages starting with the first page of the disclosure. This numbering will be used to direct the Examiner to specific portions of the provisional patent application. Additionally, as the provisional patent application is comprised of a series of manuscripts,

treatment is shown on page 78 of the provisional patent application (Figures 4 and 5 of the Hashimoto manuscript marked "In preparation") and Figures 6A and 6B and Example 8 of the instant specification.

Methods and reagents that inhibit formation of NACP/α-synuclein aggregates in vitro should be useful in the inhibition of the formation of aggregates in vivo as the aggregates are formed essentially of Aβ and/or NACP/α-synuclein. The page proofs of the *Brain Research* paper that comprise the first section of the provisional patent application (pages 1-6) teaches a number of conditions under which NACP/α-synuclein is fibrillated in vitro and the relevance of such information to neurodegenerative disease, specifically Lewy body disease. Specifically, the thioflavine-S staining of the aggregates formed in vitro were similar to that seen in Lewy bodies and distrophic brains (see page 4 of the provisional patent application, Figure 3 of the page proofs). This strongly suggests that plaques may be formed of NACP/α-synuclein alone as the protein is clearly capable of forming aggregates alone. This is also discussed in the specification of the instant application on page 7, ln 25 to pg 8, ln 8. It is well known that plaques play an important role in the pathology of neurodegenerative disease; therefore, inhibition of formation of plaques should ameliorate disease. All of the limitations of claim 4 are supported by both the provisional patent application and the instant specification.

The remaining claims are also supported by both the provisional patent application and the specification. Methods of induction of oxidative stress in claims 5-6 and 11 (which is now incorporated into claim 1) are supported generally in the Hashimoto et al. *NeuroReport* accepted manuscript (pages 17-28 of the provisional) and specifically on pages 22-23 in the Results sections entitled "Ferric iron stimulates aggregation of NACP/α-synuclein" and "Aggregation of NACP/α-synuclein may be associated with iron-catalyzed oxidation." The data from the experiments are shown in Figures 1 and 2 of that manuscript (page 28 of the provisional patent application). The same data are shown in Figures 2A and 2B of the instant application and discussed on page 5, lines 24-29. Thus the

page proofs and publications, the sections of the provisional patent application will be referred to in light of the titles and headings of each of the sections.

The newly amended claim 4 recites a method for testing potential treatments of neurodegenerative disease comprising inducing protein aggregation by oxidative stress in a first and second sample. The method of forming aggregates using oxidative stress is taught in the provisional patent application on pages 17-28 in the submitted manuscript entitled "Oxidative stress induces amyloid-like aggregate formation of NACP/α-synuclein in vitro," by Makoto Hashimoto et al. More specifically, methods for inducing aggregation are discussed in the results section, pages 22-23 of the provisional patent application (pages 6-7 of the manuscript) and all of the figures. Example 1 of the specification of the instant application (page 22, ln 4ff) teaches the same experiments and results. These methods are directed to aggregation of purified protein in vitro. Oxygen free radicals and oxidizing agents can have effects through membranes, causing intracellular damage. Therefore, the α-synuclein for use in the method of the instant invention need not be purified in a test tube. It may exist in cells or tissues.

Inclusion of an inhibitor of aggregation in a sample is within the ability of those skilled in the art.

Methods of comparing the level of aggregation in samples is taught both in the provisional patent application and the specification of the instant application. Methods include the use of western blots to detect a decrease in mobility caused by aggregation of the sample, and direct observation of the aggregates by microscopy. In the provisional patent application, the figures on pages 2 and 3 demonstrate aggregation as detected by western blot and microscopy, respectively. Page 28 of the provisional patent application demonstrates aggregation of α-synuclein by western blot specifically in response to oxidative stress. Similar data are presented in other portions of the provisional patent application. Example 5 in the specification (p 26, ln 1ff) and Figures 1 and 2 show western blot aggregation assays. Quantitative analysis of the inhibition of aggregation by a

limitations of the claims are fully supported.

Claim 7 teaches the treatment as being a non-amyloidogenic protein that modulates aggregation of NACP/α-synuclein. Claim 8 teaches the non-amyloidogenic protein being β-synuclein. Pages 55-78 of the provisional patent application, the Hashimoto et al manuscript in preparation, demonstrates that β-synuclein can inhibit aggregation of NACP/α-synuclein in vitro, supporting claim 8. This is most clearly seen in Figure 5 and discussed specifically on page 64 of the provisional patent application (page 10 of the manuscript). Figures 1 and 2 of the same manuscript (pages 74-75), which are identical to Figures 3-5 in the instant application teach how to make the non-amyloidogenic β-synuclein into an amyloidogenic protein. Discussion of the figures is provided in each of the documents as well. Therefore, by removing these sequences from NACP/α-synuclein, one would expect to generate a non-amyloidogenic protein based on the homology of the two proteins, providing multiple proteins that could be used in claim 7. Thus, claims 7 and 8 are supported by the specification and the provisional patent application.

Claims 12 and 13 are supported by the Hashimoto et al *BBRC* reference (pages 110-117 of the provisional patent application) that demonstrates that the regulation of the expression of α- and β-synuclein is different during megakaryocyte differentiation. Therefore, one would expect a difference in the regulation of the expression in neuronal cells. This is also strongly suggested by the fact that a concurrent increase in β-synuclein expression is not seen with the abnormal increase in the expression of α-synuclein that results in a disease state. A variety of molecules are known that selectively regulate transcription. Using the method of claim 4, a number of such agents can be tested for promoting expression of anti-amyloidogenic proteins or other factors. The changes that cells undergo during aggregation of α-synuclein are well known. Thus, claims 12 and 13 are supported by the provisional patent application and the instant application.

Claims 14 and 15 teach the use of the assay method in cells. A number of cells that express NACP/α-synuclein are known. Not all such cell lines are neuronal.

Megakaryocytes express both α- and β-synuclein as mentioned above. Methods of primary culture are well known. Thus, claims 14 and 15 are supported by the provisional patent application and the instant application.

In view of the detailed discussion and multiple references cited in the discussion above that the rejection of the claims under 35 U.S.C. 112, paragraph 1 is traversed.

*Rejection under 35 U.S.C. §112, ¶1.* The Examiner has rejected to claims 4-8 and 11-13 under 35 U.S.C. 112, paragraph 2 for lacking clarity. The Applicants have amended the claims as set forth above to increase clarity. The limitation of exposure to an oxidizing agent has been added to claim 4 and the term "inducing oxidative stress" has been removed as the Applicants wish to claim both in vitro and in vivo assays as taught in the instant application. The term "expression" has been removed from claims not directed to the use of cells. Therefore the rejection under 35 U.S.C. §112, ¶1 is traversed.

*Priority.* The Examiner questions if the matter claimed in the instant application is supported by the provisional patent application. In view of the analysis above, the Applicants submit that all of the claimed matter is supported in the provisional patent application and that the filing date of the provisional patent application should be considered the proper priority date of the instant application.

*Rejections under 35 U.S.C. §102.* The Examiner has rejected claim 4 as being anticipated by Biere, USP 6,184,351, filed on September 24, 1999 and issued on February 6, 2001. The filing date of Biere is substantially later than the priority date of the instant application. Therefore, it is not available as prior art and the rejection of claim 4 under 35 U.S.C. §102 is traversed.

The Examiner has rejected claims 4, 7-8 and 12-13 under 35 U.S.C. §102(b) as being anticipated by Jensen et al. The Applicants have included the limitation of claim 11

into the newly amended claim 4. As claim 11 is not included in the rejection of the claims in view of Jensen, the rejection of claim 4 is traversed. As the remaining claims 7-8 and 12-13, as well as the newly added claims 14-15, are dependent either directly or indirectly on claim 4, those claims are also allowable. Therefore the rejection under 35 U.S.C. §102(b) is traversed.

*Rejections under 35 U.S.C. §103(b).* The Examiner has rejected claims 5-6 and 11 for obviousness over Hashimoto et al., NeuroReport 10:717-721, 1999 alone or alternatively in view of Biere et al or Jensen et al. The Applicants have demonstrated that all of the claims of the instant application are supported by the provisional patent application on which it is based. Therefore, the Hashimoto and Biere references, both references under 102 only after the priority date of the instant application are not available as prior art. Moreover, the submitted manuscript of the Hashimoto reference comprises pages 17-28 of the provisional patent application. Therefore, all of the matter in the Hashimoto reference is taught by the instant application. As the Jensen reference, the only reference that qualifies as prior art in this rejection must be combined with a second reference to make the claims obvious, no rejection can be made. Therefore, the rejection of claims 5-6 and 11 under 35 U.S.C. §103(a) is traversed.

## FEES

It is believe that there are no fees due with this response. However, if a fee is due, the Commissioner is hereby entitled to charge Deposit Account 02-4070 referencing case number 6627-PA9013.

## CONCLUSIONS

In view of the above amendments and remarks, it is respectfully submitted that all grounds of rejection and objections have been traversed. The Examiner is therefore

respectfully requested to allow the case to proceed to issuance with the claims 4-8 and 12-15 as shown above.

Should the Examiner believe that prosecution of this application might be expedited by further discussion of the issues, she is cordially invited to telephone the undersigned agent for Applicant, collect, at the telephone number listed below.

Respectfully submitted,

Dated: February 2, 2004

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